FORM PTO-1390 (Modified) (REV 5-93) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 084335/0127

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5): Unassigned 09/744237

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP99/03859	July 16, 1999	July 17, 1998

TITLE OF INVENTION
TESTIS-SPECIFIC DIFFERENTIATION-REGULATORY FACTOR

APPLICANT(S) FOR DO/EO/US
Takashi SUGIHARA, Renu WADHWA, Sunil C. KAUL, Youji MITSUI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.

3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   - ☒ has been transmitted by the International Bureau.
   - ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   - ☐ have been transmitted by the International Bureau.
   - ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - ☒ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information: Paper copy of Sequence listing (20 pages)

534 Rec'd PCT/PTO 05 JAN 2001

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
Unassigned 09/745237	PCT/JP99/03859	084335/0127

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO.....$860.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482).....$690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2) $710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor International search fee (37 CFR 1.445(a)(2)) paid to USPTO $1,000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than 20 Months from the earliest claimed priority date (37 CFR 1.492(e))		

Claims	Number Filed		Included in Basic Fee		Extra Claims		Rate		
Total Claims	15	-	20	=	0	×	$18.00	$0.00	
Independent Claims	2	-	3	=	0	×	$80.00	$0.00	
Multiple dependent claim(s) (if applicable)							$270.00		
TOTAL OF ABOVE CALCULATIONS =								$860.00	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).								$0.00	
SUBTOTAL =								$860.00	
Processing fee of $130.00 for furnishing English translation later the 20 months from the earliest claimed priority date (37 CFR 1.492(f). +									
TOTAL NATIONAL FEE =								$860.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +									
TOTAL FEES ENCLOSED =								$860.00	
							Amount to be: refunded	$	
							charged	$	

a. ☒ A check in the amount of $860.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 19-0741 in the amount of $0.00 to the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0741. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

NAME STEPHEN A. BENT

REGISTRATION NUMBER 29,768

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Takashi SUGIHARA et al.

Title: TESTIS-SPECIFIC DIFFERENTIATION-REGULATORY FACTOR

Appl. No.: Unassigned

Filing Date: January 5, 2001

Examiner: Unassigned

Art Unit: Unassigned

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the present Application, Applicants respectfully request that the above-identified application be amended as follows:

In the Claims:

Claim 4, line 1, delete "any one of claims 1 to 3" and insert --claim 1--

Claim 7, line 1, delete "the" and insert --a--

line 1, delete "of any one of claims 1 to 3"

line 2, delete "the" and insert --a--

line 2, delete "of claim 6" and insert --comprising DNA encoding the protein of claim 1--

line 3, after "and", insert --then--

Claim 8, lines 1 and 2, delete "any one of claims 1 to 3" and insert --claim 1--

Please add the following new claims:

10. A DNA encoding the protein of claim 2.

11. A DNA encoding the protein of claim 3.

12. A method of producing a protein comprising the steps of culturing a transformant comprising DNA encoding the protein of claim 2 and then collecting the expressed protein from said transformant or the culture supernatant thereof.

13. A method of producing a protein comprising the steps of culturing a transformant comprising DNA encoding the protein of claim 3 and then collecting the expressed protein from said transformant or the culture supernatant thereof.

14. An antibody binding to the protein of claim 2.

15. An antibody binding to the protein of claim 3.

REMARKS

Applicants respectfully request that the foregoing amendments to Claims 4, 7 and 8, and new Claims 10 through 15, be entered in order to avoid this application incurring a surcharge for the presence of one or more multiple dependent claims.

Respectfully submitted,

Date January 5, 2001

By _____

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DESCRIPTION

TESTIS-SPECIFIC DIFFERENTIATION-REGULATORY FACTOR

Technical Field

The present invention relates to a protein and its gene involved in the differentiation of testicular cells and belongs to the field of bioscience, specifically, developmental biology.

Background Art

In the developmental process, reproductive cells carry out spermatogenesis via a differentiation process that includes meiosis. This differentiation process is different from that of somatic cells and consists of three main steps. The first step is the proliferation of spermatogenous cells and differentiation into primary spermatocytes. The second is the meiosis of primary spermatocytes, and the third is the transformation into sperms.

Owing to the progress in Molecular Biology, recent years have seen the isolation of several genes specifically expressed in these stages. For example, Hox-1.4 (Propst, F. et al. (1988) Oncogene 2:227-33), ferT (Sarge, K. D. et al. (1994) Biol Reprod 50:1334-1343) of the HSP70 family, and TESK1 (Toshima, J. et al. (1995) J. Biol. Chem. 270:31331-31337) that is a serine-threonine kinase, have been reported as genes specific to primary spermatocytes. However, still very little is known about the biological and physical roles of their gene products.

Genes expressing specifically in the differentiation process of reproductive cells carry a fundamental and vital role that decides the fate of those cells, and thus, defects in these genes are considered to be a cause of diseases such as infertility. Therefore, genes expressing specifically in the differentiation process of reproductive cells are recently gaining wide attention as targets in the development of pharmaceutical drugs. Such drugs can be used for the prevention and treatment of diseases such as infertility caused by defects in reproductive cell differentiation.

Disclosure of the Invention

The present invention provides a novel protein relating to the differentiation of testicular cells, and the encoding gene. It also provides a vector and transformant used for, for example, producing the protein, and a method of producing the protein. The present invention also provides an oligonucleotide used for the isolation and the detection of the gene of the invention.

The inventors were evaluating the expression of genes encoding unknown proteins that trigger cell death when, irrelevant to their original aim, they unexpectedly succeeded in isolating a novel gene specifically expressed in the testis. When the databases were searched for the isolated gene, it was found to be a novel gene that did not have a significant homologous gene. Structural analysis of the protein encoded by the gene showed that it had in part a structure similar to the metal-binding site of metallothionein, which is known to be a metal-binding factor. Expression analysis in tissues revealed that the gene is extremely specific to the testis, especially to primary spermatocytes. The expression was not seen in the testis of infertile mice. Analysis of the human and mouse chromosomal locations showed that the gene was located in the same site as the gene locus that is known to be defective in infertile mice. Results of these analyses suggest that the protein encoded by the isolated gene is involved in regulating the differentiation of the testis.

The present invention relates to a novel protein involved in the regulation of testicular differentiation having a metal-binding site, and the gene thereof, more specifically:

(1) a protein comprising the amino acid sequence of SEQ ID NO: 4 or 5;

(2) a protein which comprises an amino acid sequence in which one or more amino acids in the amino acid sequence of SEQ ID NO: 4 or 5 have been replaced, deleted, and/or added, and which is functionally equivalent to the protein of (1);

(3) a protein which is encoded by a DNA hybridizing to the DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3, and

which is functionally equivalent to the protein of (1);

(4) a DNA encoding the protein of any one of (1) to (3);

(5) a vector comprising the DNA of (4);

(6) a transformant comprising the DNA of (4) in an expressible manner;

(7) a method of producing the protein of any one of (1) to (3) comprising the steps of culturing the transformant of (6), and collecting the expressed protein from said transformant or the culture supernatant thereof;

(8) an antibody binding to the protein of any one of (1) to (3); and,

(9) a DNA specifically hybridizing to a DNA comprising the nucleotide sequence of any one of SEQ ID NOs: 1 to 3, and comprising at least 15 nucleotides.

The present invention provides the protein Tesmin, which may regulate the differentiation of spermatogenous cells into primary spermatocytes, and the gene thereof.

The inventors isolated two types of Tesmin cDNA of mouse origin arising possibly from splicing differences in the transcriptional process. The nucleotide sequences of these cDNAs are shown in SEQ ID NOs: 1 and 2, and the amino acid sequence of the protein encoded by these cDNAs in SEQ ID NO: 4. The nucleotide sequence of human Tesmin cDNA also isolated by the inventors is shown in SEQ ID NO: 3, and the amino acid sequence of the protein encoded by the cDNA is shown in SEQ ID NO: 5.

As shown in SEQ ID NOs: 1 and 2, mouse-derived Tesmin cDNA has an ORF encoding a protein comprising 295 amino acids. On the other hand, human-derived Tesmin cDNA has an ORF encoding a protein comprising 299 amino acids, as shown in SEQ ID NO: 3. SDS-PAGE analysis of the *in vitro* translational product of mouse Tesmin using ^{35}S-labeled methionine showed that mouse Tesmin protein had a molecular weight of 32.5 kDa (Fig. 3).

Among the tissues within the body, both mouse and human Tesmin genes were expressed only in the testis, as revealed by Northern blot analysis and RT-PCR (Figs. 1 and 2). RT-PCR analysis showed

that Tesmin gene is hardly expressed in the immature testis up to day 8 following birth, but the expression increases from day 12 when the sperm differentiation starts, and its high expression stabilizes from day 18. In the W/Wv mouse known as an infertile mouse that lacks the growth factor receptor "c-kit" gene, Tesmin gene expression was hardly seen even in the matured testis of day 52 following birth (Fig. 4). These facts suggest that the Tesmin protein is involved in the differentiation of the testis. The Tesmin protein and its gene can be applied, for example, in the treatment of infertility.

The Tesmin protein of the invention can be prepared by incorporating DNA encoding the protein (e.g., DNA comprising the nucleotide sequence of any one of SEQ ID NO: 1 to 3) into a suitable vector, introducing this into a suitable host cell, and purifying the protein from the transformant obtained. The protein of the present invention can also be prepared as a recombinant protein made using genetic engineering techniques by culturing cells transformed with DNA encoding the Tesmin protein, as mentioned later. The natural protein can be isolated from testicular tissues by methods well known to one skilled in the art, for example, the affinity chromatography later described, using an antibody that binds to the Tesmin protein.

A skilled artisan can prepare not only a natural Tesmin protein, but also a modified protein functionally equivalent to the natural protein by, for example, suitably performing amino acid substitution of the protein using known methods. Amino acid mutations of a protein can occur spontaneously, too. Therefore, the protein of the invention includes a mutant in which the amino acid sequence of the natural protein was mutated by, for example, replacing, deleting, or adding one or several amino acids, and which is functionally equivalent to the natural protein. Methods well known to a skilled artisan for modifying amino acids are, for example, PCR-mediated site-specific-mutation-induction system (GIBCO BRL, Gaithersburg, Maryland), oligonucleotide-mediated site-specific-mutagenesis (Kramer, W. and Fritz, HJ (1987) Methods in Enzymol. 154:350-367), the Kunkel method

(Methods Enzymol. 85:2763-2766 (1988)), and so on. The number of amino acids mutated is normally within ten amino acids, preferably within six amino acids, and more preferably within three amino acids.

Herein, "functionally equivalent" means that the mutant protein has a biochemical and/or biological activity equivalent to the natural protein. As such activities, for example, the binding activity between the protein and metal, and the testicular cell differentiation-inducing activity can be given.

The metal-binding activity can be detected, for example, as follows. First, the recombinant Tesmin protein is EDTA-treated to remove heavy metals that may be bound to the Tesmin protein. Next, EDTA is removed by gel filtration, and then, the heavy metals (for example, Zn^{2+}, Cd^{2+}, Cu^{2+}, etc.) to be examined are added and reacted with the recombinant Tesmin protein. After reacting, the presence or absence of a metal bond is detected as CD spectra using a CD spectropolarimeter (J-500C by Jasco) (refer Presta A. et al., Eur. J. Biochem Jan 15; 227(1-2):226-240).

The testicular cell differentiation-inducing activity can be detected, for example, as follows. First, spermatogoniums, spermatogenous cells, and primary spermatocytes are isolated from mouse testis by centrifugation. Next, Tesmin gene is incorporated into an expression vector (e.g., pBK-CMV vector, Stratagene), and the gene incorporated is introduced to cells isolated by lipofectAMINE (GIBCO BRL). After culturing the cells from a few hours to a few days, the expression of a genetic marker that identifies the differentiation stage (e.g., MEG1, ssH2B, etc.) is verified by the RT-PCR method.

The hybridization technique (Sambrook, J et al., Molecular cloning 2nd ed. 9.47-9.58, Cold Spring Harbor Lab. press, 1989) is well known to a skilled artisan as an alternative method for isolating a functionally equivalent protein. In other words, it is a general procedure for a skilled artisan to isolate DNA having a high homology to the whole or part of the DNA encoding the mouse or human Tesmin protein (a DNA comprising the nucleotide sequence of any one of SEQ ID NOs: 1 to 3) and to obtain a protein functionally

equivalent to the mouse or human Tesmin protein from the isolated DNA. Therefore, the protein of the present invention also includes a protein encoded by DNA hybridizing to DNA encoding the mouse or human-derived Tesmin protein, which is functionally equivalent to these proteins. When isolating the hybridizing DNA from other organisms, there is no restriction as to the organisms used, although testicular tissues from, for example, rats, rabbits, and cattle are suitable for the isolation. DNA isolated by hybridization techniques usually has a high homology to DNA encoding the mouse- and human-derived Tesmin protein (DNA comprising the nucleotide sequence of any one of SEQ ID NOs: 1 to 3). "High homology" means, a sequence identity at the amino acid level of at least 40% or more, preferably 60% or more, more preferably 80% or more, and even more preferably, 95% or more. The homology of a sequence can be calculated, for example, by the method described in Proc. Natl. Acad. Sci. USA (1983) 80:726-730.

An example of hybridization conditions (stringent) for isolating a DNA high in homology is as follows. Namely, after conducting a prehybridization at 68°C for 30 min or more using the "Rapid-hyb buffer" (Amersham LIFE SCIENCE), a labeled probe is added, and hybridization is done by incubating at 68°C for 1 hr or more. After that, washing is done three times within 2x SSC/0.01% SDS for 20 min at room temperature, and next, three times within 1x SSC/0.1% SDS, at 37°C for 20 min, followed by, two times within 1x SSC/0.1% SDS, at 50°C for 20 min.

This invention also provides a DNA encoding the Tesmin protein. The DNA of the present invention includes genomic DNA, synthetic DNA, and such, as well as cDNA, as long as such DNA encodes the Tesmin protein of the invention. The DNA of the invention can be used, for example, for producing recombinant proteins. Namely, the recombinant proteins can be prepared by inserting the DNA of the invention (e.g., SEQ ID NOs: 1 and 2) into a suitable expression vector, introducing this into a suitable cell, culturing the resulting transformant, and purifying the protein expressed. Cells used for the production of recombinant proteins are, for example, mammalian cells such as COS cells, CHO cells, and NIH3T3

cells; insect cells such as Sf9 cells; yeast cells; and *E.coli*, but there is no restriction as to the cells used. The vector for expressing the recombinant protein within cells varies according to the host cell, and, for example, pcDNA3 (Invitrogen), and pEF-BOS (Nucleic Acids. Res. 1990, 18 (17), p5322) and such are given as vectors for mammalian cells, Bac-to-BAC baculovirus expression system (GIBCO BRL) and such for insect cells, Pichia Expression Kit (Invitrogen) and such for yeast cells, and pGEX-5X-1 (Pharmacia) and QIAexpress system (Qiagen) and such for *E.coli*. Vectors can be introduced into hosts for example, by the calcium phosphate method, DEAE dextran method, the method using cationic liposome DOTAP (Boehringer Mannheim), electroporation method, calcium chloride method, etc. Transformants can be cultured according to their properties using methods well known to skilled artisans. Recombinant proteins can be purified from transformants by methods well known to skilled artisans, for example, the methods described in reference "The Qiaexpressionist handbook, Qiagen, Hilden, Germany."

The present DNA can be used for gene therapy of diseases caused by mutations of the gene. The Tesmin gene especially may be the causative of the genetic disease of infertile mice, and therefore, is expected to be applied in the gene therapy of infertility. When using for gene therapy, the DNA of the invention is inserted into, for example, a viral vector such as an adenovirus vector (e.g. pAdexLcw) and a retrovirus vector (e.g. pZIPneo), or a non-viral vector, and administered to a target site of the body. The method of administration may be *ex vivo* or *in vivo*.

The present invention also provides an antibody that binds to the protein of the invention. The antibody of the present invention includes polyclonal antibodies and monoclonal antibodies. These antibodies can be prepared by following methods well known to skilled artisans. Polyclonal antibodies can be made by, for example, obtaining the serum of small animals such as rabbits immunized with the protein (or a partial peptide) of the present invention, and purifying by, for example, ammonium sulfate precipitation, a protein A or protein G column, etc. Monoclonal

antibodies can be made by immunizing small animals such as mice with the protein (or a partial peptide) of the present invention, excising the spleen from the animal, homogenizing the organ into cells, fusing the cells with mouse myeloma cells using a reagent such as polyethylene glycol, selecting clones that produce antibodies against the protein of the invention from the fused cells (hybridomas), transplanting the obtained hybridomas into the abdominal cavity of a mouse, and collecting ascites from the mouse. The obtained monoclonal antibodies can be purified by, for example, ammonium sulfate precipitation, a protein A or protein G column, etc. The antibody thus prepared can be applied for antibody therapy and such, other than for the purification and detection of the protein of the invention. When administrating the antibody to humans with the aim of antibody therapy, humanized antibodies are effective in decreasing immunogenicity. Antibodies can be humanized by, for example, cloning the antibody gene from monoclonal antibody producing cells and using the CDR graft method which transplants the antigen-recognition site of the gene into a known human antibody. Human antibodies can also be prepared like ordinary monoclonal antibodies by immunizing a mouse whose immune system has been replaced by a human immune system with the protein of the invention.

This invention also provides a DNA specifically hybridizing to DNA encoding the Tesmin protein and comprising at least 15 nucleotides. The term "specifically hybridizing" as used herein indicates that cross-hybridization does not significantly occur with DNA encoding proteins other than the Tesmin protein, under the usual hybridization conditions, preferably under stringent hybridization conditions. Such DNA can be used as a probe for detecting or isolating DNA encoding the Tesmin protein, or as a primer for amplification. Tesmin gene is expressed only in the testis, and even in the testis, it is expressed for a limited period. Therefore, the DNA can be used as a testis differentiation marker (a test drug). Also, there is a possibility that the Tesmin gene is the causative gene of the genetic disease of infertile mice, and therefore, the DNA may be used for the testing of infertility.

Brief Description of the Drawings

Figure 1 is an electrophoretic photograph showing the results of Northern blot analysis of Tesmin gene expression in various mouse tissues.

Figure 2 is an electrophoretic photograph showing the results of Northern blot analysis of Tesmin gene expression in various human tissues.

Figure 3 is an electrophoretic photograph showing the results of the molecular weight detection of mouse Tesmin protein expressed by *in vitro* translation.

Figure 4 is an electrophoretic photograph showing the results of Northern blot analysis of Tesmin gene expression in the testis of ICR strain mouse at day 4, 8, 12, 18, and 42 following birth, and in the day 56 testis of W/Wv strain mouse. "MEG1" and "ssH2B" were used as testis differentiation markers. "GAPDH" was used as the control.

Figure 5 is a photomicrograph showing the results of detection of Tesmin gene expression in testicular tissues by *in situ* hybridization.

Figure 6 is a photomicrograph and schematic diagram of the chromosomal location showing the results of the detection of Tesmin gene location in the mouse chromosome using a probe specific to the Tesmin gene.

Figure 7 is a photomicrograph and schematic diagram of the chromosomal location showing the results of the detection of Tesmin gene location in the human chromosome using a probe specific to the Tesmin gene.

Figure 8 is a photomicrograph showing the intracellular localization of the complete Tesmin protein and its deletion mutant.

Figure 9 shows the results of the detection of the Tesmin protein (fusion protein with GST) by Western blotting using the prepared anti Tesmin antibody. Detection using anti GST antibody was also done concurrently. "IPTG+" means the protein detected by adding isopropyl-β-D-thiogalactoside (IPTG) to cDNA-introduced

E.coli to induce the expression of the recombinant protein, subjecting the cell lysate to SDS-PAGE and Western-blotting, and "IPTG-" means the protein detected in a lysate of *E.coli* to which IPTG was not added.

Best Mode for Carrying Out the Invention

The invention shall be specifically described in examples below, but it is not to be construed as being limited thereto.

Example 1: Isolation of Tesmin gene fragment using RT-PCR

The expression of the novel substance WF-1 (a function-unknown novel gene comprising 1700 bp) in each organ was analyzed by the RT-PCR method. Specifically, total RNA was extracted from the brain, liver, spleen, kidney, heart, and testis of ICR strain mice (Clea Japan) using ISOGEN (NIPPON GENE). After denaturing RNA at 65°C, cDNA was prepared using reverse transcriptase: superscript 2 (GIBCO BRL). Using cDNA from each organ, and the oligo primers for WF-1 amplification described in SEQ ID NOs: 6 and 7, PCR reaction was conducted for 32 cycles of 94°C for 1 min, 58°C for 2 min, and 72°C for 3 min. The control GAPDH was amplified by PCR using the oligo primers described in SEQ ID NOs: 8 and 9 under the condition of 30 cycles of 94°C for 1 min, 58°C for 2 min, and 72°C for 3 min. As a result, a gene specifically expressed only in the testis was unexpectedly found through a detection using oligo primers for WF-1 amplification described in SEQ ID NOs: 6 and 7. This cDNA fragment was isolated, and the gene encoded by the cDNA was named "Tesmin" (first named "Testin," but later changed to "Tesmin").

Example 2: Cloning and sequencing of mouse Tesmin cDNA

The sequence of the above cDNA fragment was determined by the dideoxy chain termination method and analyzed by the ABI377 auto sequencer. As a result of a database search for the determined sequence, this sequence was revealed to be a novel gene that did not have a homology to genes in the databank. This cDNA fragment was ^{32}P radio-labeled to prepare a probe, and using this, a mouse

testis library was screened. As a result, a clone having an approximately 1.7 kb length was obtained.

Moreover, 5'-RACE was conducted to determine the 5' end sequence. In 5'-RACE, three antisense primers specific to the Tesmin gene, namely, SP1 (SEQ ID NO: 10), SP2 (SEQ ID NO: 11), and SP3 (SEQ ID NO: 12), and mouse testis-derived 5'-Marathon RACE cDNA were used. 5'-RACE method was conducted following the "Marathon-ReadyTM cDNA kit (mouse testis)" (Clontech) protocol. The whole nucleotide sequence of Tesmin cDNA obtained is shown in SEQ ID NOs: 1 (2241 bp) and 2 (1861 bp). These two cDNAs are thought to be splicing variants arising from a difference in splicing at the point of transcription.

When the database was searched using these cDNA sequences, no sequence comprising a significant homology was found in the databank. These cDNAs encode the same protein comprising 295 amino acids (pI-7.64), and no significantly homologous proteins were found in the protein database as well.

Example 3: Cloning and sequencing of human Tesmin cDNA

Mouse Tesmin plasmid (a plasmid in which the Tesmin gene has been inserted into the pBluescript2 vector) was cleaved by SphI-SalI, and this 1.7 kb gene fragment was used as a probe to screen the cDNA library prepared by human testis mRNA. Hybridization was done using the "Rapid-hyb buffer" (Amersham LIFE SCIENCE) under the following conditions: (i) a prehybridization at 60°C for 30 min, (ii) addition of the labeled probe, and (iii) hybridization by incubating at 60°C for 2 hr. After that, washing is done three times within 2x SSC, 0.01% SDS for 20 min at room temperature, and next, three times within 1x SSC, 0.1% SDS, at 37°C for 20 min, followed by, two times within 1x SSC, 0.1% SDS, at 50°C for 20 min.

The nucleotide sequence of thus obtained human Tesmin cDNA is shown in SEQ ID NO: 3. Database search for the determined nucleotide sequence was done but there were no homologous sequences within the databank, similar to the mouse cDNA. The obtained human cDNA had four amino acids more than mouse Tesmin

and encoded a protein comprising 299 amino acids (pI-7.71). No significant homology was found in the protein database as well. However, as a result of amino acid sequence analysis by BLAST, the mouse and human Tesmins were found to be cysteine-rich proteins partially having the structure very similar to the metal-binding domain of the metallothionein family.

Metallothionein expression in the liver is induced by heavy metals, and metallothionein is known as a protein that neutralizes metallic poison. However, in the testis, the metallothionein gene is constantly expressed and is not induced by metals. Therefore, it was thought to play some vital roles other than metal binding in the testis. Recent findings showed that the estrogen receptor, which is a zinc-finger transcription factor and a receptor protein, and metallothionein conduct metal transfer *in vitro* (Cano-Gauci, D. and Sarkar, B. (1996) FEBS Lett 386 (1):1-4). Therefore, the metal-binding site of metallothionein is thought to play a vital role in the regulation of transcription factors. The "Cys-X-Cys-X-X-X-X-X-X-X-X-Cys-X-Cys (where X is an arbitrary amino acid)" sequence having a cysteine structure in the amino acid sequence is thought to be vital for metal binding in the metallothionein family.

This cysteine structure (in mouse, from the 157th to the 171st positions, in human, from the 161st to the 175th positions) was conserved in Tesmin too. However, the metallothionein family members known up to now were relatively low-molecular comprising 60 to 70 amino acids, whereas Tesmin was comparatively longer (mouse: 295 amino acids, human: 299 amino acids). Domain search by PROSITE revealed that mouse Tesmin had a N-myristylation site and a casein kinase 2 phosphorylation site. Human Tesmin had a cAMP and cGMP-dependent kinase phosphorylation site, a protein kinase C phosphorylation site, a N-myristylation site, and a N-glycosylation site. Other than those, a cAMP and cGMP-dependent protein kinase phosphorylation site and a protein kinase phosphorylation site were also present.

Domain search by BLOCKS revealed sites common to mouse and human Tesmins. Namely, "high potential iron-sulfate protein"

(from 87th to 103rd positions in mouse, from 87th to 103rd positions in human), "Adenodoxin family" (iron-sulfate binding region) (from 177th to 194th positions in mouse, from 181st to 198th positions in human), "Alpha-2-mavrogloburin family thiolester region" (from 243rd to 252nd positions in mouse, from 247th to 256th in human), "Arrestins proteins" (from 267th to 277th positions in mouse, from 271st to 281st positions in human), "Ribosomal protein L14 proteins" (from 5th to 26th positions in mouse, from 5th to 26th positions in human), "Cooper amine oxidase topaquinone proteins" (from 81st to 109th positions in mouse, from 81st to 109th positions in human), and "VFWC domain proteins" (from 13th to 19th positions and from 105th to 113th positions in mouse, from 105th to 113th positions in human) were confirmed in mouse and human Tesmins. When sequence features were analyzed by PRINTS, both mouse and human Tesmins had a "Rhodopsin-like GPCR superfamily signature" (from 93rd to 117th positions, from 231st to 252nd positions, and from 232nd to 253rd positions in mouse, from 43rd to 67th positions and from 236th to 257th positions in human).

Example 4: Transcription and translation *in vitro*

In vitro translation was done to verify the open reading frame anticipated in mouse Tesmin. Specifically, the cDNA pBluescript-Tesmin cloned from the testis was transcribed and translated for one hour *in vitro* using the rabbit reticulocyte lysate (Promega) to which L-[^{35}S] methionine has been added. Translation products were separated by SDS-PAGE, and detected by autoradiography. As a result, a protein of approximately 32.5 kDa was detected (Fig. 3). This product coincided well with the size of the protein thought to be the ORF within the mouse Tesmin sequence.

Example 5: Preparation of recombinant Tesmin

The open reading frame of mouse Tesmin cDNA was amplified by a PCR reaction using sense (SEQ ID NO: 19) and antisense (SEQ ID NO: 20) primers having an EcoRI site. The fragment amplified by the PCR reaction was cloned to the pGEM-T vector to verify its

precise sequence. Next, it was cleaved by EcoRI-EcoRI, and finally cloned to pGEX-2TK vector that produces GST fusion protein. Tesmin product cloned into pGEX-2TK was gene transfected into *E.coli* JM109, induced using IPTG 0.2 mM at 37°C for 3 hr, and the *E.coli* lysate was separated by SDS-PAGE, and detected by Western blotting using the GST antibody. As a result, a 58.5 kDa protein comprising a GST fusion portion with a molecular weight of 26 kDa was synthesized (Fig. 8 left). The recombinant protein had the same size expected by the presumed size of the molecule, similar to the result of *in vitro* translation.

Example 6: Northern blot analysis

A membrane loaded with 2 μg/lane of various mouse and human tissue mRNA was purchased (Clontech laboratories, Palo alto, CA), and Northern blot analysis was conducted. The probe was a 1.7 kb gene fragment made by cleaving mouse Tesmin plasmid (a plasmid in which the Tesmin gene has been inserted into pBluescript2 vector) with SphI-SalI. Hybridization was done using the "Rapid-hyb buffer" (Amersham LIFE SCIENCE) under the following conditions: (i) a prehybridization at 68°C for 30 min,(ii) addition of the labeled probe, and, (iii) hybridization by incubating at 68°C for 2 hr. Next, washing is done three times within 2x SSC, 0.01% SDS for 20 min at room temperature, and next, three times within 1x SSC, 0.1% SDS, at 37°C for 20 min, followed by, two times within 1x SSC, 0.1% SDS, at 50°C for 20 min. Detection was done by autoradiography. Similar to the results of RT-PCR, Tesmin gene expression was detectable in the testis only, and gene expression was seen at the 2.4 kb and 2.0 kb locations in mouse (Fig. 1) and in just the 2.4 kb location in human (Fig. 2).

Example 7: Involvement in the differentiation of reproductive cells

Total RNA was extracted from the testis of ICR strain mouse at day 4, 8, 12, 18, and 42 following birth, and from the day 56 testis of W/Wv strain mouse (Japan SLC; type WBB6F1-W/Wv known as an infertile mouse deficient of the mouse growth factor S1 receptor

c-kit gene; refer Chabot, B. et al. (1988) Nature 335 (6185):88-9, Yoshinaga, K. et al. (1991) Development 113 (2):689-99) using ISOGEN (NIPPON GENE). After denaturing this RNA at 65°C, cDNA was prepared using reverse transcriptase: superscript 2 (GIBCO BRL). Tesmin gene was amplified using the oligo primers described in SEQ ID NOs: 6 and 7, under the conditions of 35 cycles of 94°C for 1 min, 58°C for 2 min, and 72°C for 3 min. The control GAPDH gene was amplified using the oligo primers described in SEQ ID NOs: 8 and 9, under the conditions of 30 cycles of 94°C for 1 min, 58°C for 2 min, and 72°C for 3 min. For the MEG1 PCR reaction, the oligo primers described in SEQ ID NOs: 13 and 14 were used, under conditions of 35 cycles of 94°C for 1 min, 58°C for 2 min, and 72°C for 3 min. For the ssH2B reaction, the oligo primers described in SEQ ID NOs: 15 and 16 were used, under the conditions of 35 cycles of 94°C for 1 min, 58°C for 2 min, and 72°C for 3 min. Marker MEG1 expresses when spermatogenous cells divide into primary spermatocytes (Don, J. and Wolgemuth, D.J. (1992) Aug; 3 (8):495-505), and marker ssH2B is known to express at spermatogenesis (Unni, E. et al. (1995) Biol Reprod, Oct; 53 (4):820-826).

PCR analysis showed that Tesmin gene is not expressed until day 8 following birth, having a weak expression at day 12, and taking a stable expression pattern from day 18 (Fig. 4). Tesmin gene expression pattern in the testis was similar to MEG1. Therefore, it was revealed that Tesmin expression is regulated at time points similar to MEG1.

When Tesmin expression in the W/Wv mouse was examined, it was revealed that Tesmin is not expressed in these mice. Since the W/Wv mouse is known to be an infertile mouse, the relationship between Tesmin gene and infertility was strongly suggested (Fig. 4).

Example 8: *In situ* hybridization

Labeled RNA probe was prepared from mouse Tesmin plasmid (the pBluescript2 vector in which the Tesmin gene has been inserted) using T7 and T3 polymerases and digoxigenin-dUTP. This probe was

hybridized to sliced mouse testis tissue within a solution containing 50% formamide, 10% dextran sulfate, and 2x SSC. The slide glass on which hybridization was done was incubated within a solution of anti-digoxigenin antibody bound to alkaline phosphatase, and the signal specific to hybridization was detected using the chromogenic substrate NBT/BCIP. As a result, it was verified that Tesmin is extremely specifically expressed in the testis, especially in primary spermatocytes (Fig. 5).

Example 9: Chromosomal location

Mouse P1 genomic library was obtained by PCR screening the P1 bacteriophage genomic library using a mouse Tesmin-specific sense primer (SEQ ID NO: 6) and an antisense primer (SEQ ID NO: 7). Also, human P1 genomic library was obtained using human Tesmin-specific sense primer (SEQ ID NO: 17) and antisense primer (SEQ ID NO: 18) and conducting a screening similar to mouse. The isolated P1 clones were used to examine the chromosomal localization by fluorescent *in situ* hybridization (FISH). Mouse and human P1 clone-derived DNA was labeled by nick translation using digoxigenin-dUTP, and this probe was hybridized to mouse and human primary fibroblast-derived metaphase chromosomes within a solution containing 50% formamide, 10% dextran sulfate, and 2 x SSC. The slide glass on which hybridization was done was incubated within a solution of fluorescence-labeled anti-digoxigenin antibody, and the signal specific to hybridization was detected by counter staining using 4'6'-diamino-2-phenolindol (DAPI).

As a result, the above P1 clones were found to encode the Tesmin gene since the mouse and human Tesmin-specific probes hybridized to the respective P1 clone. When DAPI staining was done using these P1 clones as probes, the 19^{th} B chromosome and the 11^{th} q13.2 chromosome were specifically labeled in mouse and human, respectively. The above results confirmed that Tesmin was located on the 19^{th} B chromosome (Fig. 6) in mouse, and on the 11^{th} q13.2 chromosome (Fig. 7) in human. The relationship between Tesmin and mouse genetic disease was examined based on these results using the Jackson Laboratory Database to find that there is a study

reporting that a mutation on the 19th B chromosome where Tesmin exists causes infertility in mice (Evans, EP. (1977) Mouse News Letter, 17). This suggests the possibility that Tesmin mutations trigger infertility in mice.

Example 10: Intracellular localization

A DNA encoding whole open reading frame of the Tesmin cDNA were prepared, using sense (SEQ ID NO: 19) and antisense (SEQ ID NO: 20) primers having an EcoRI site, and also prepared was a gene designed so that 70 amino acids are deleted from the Tesmin cDNA open reading frame, by using a sense (SEQ ID NO: 19) primer having an EcoRI site and antisense (SEQ ID NO: 21) primer having an SalI site. These genes were treated with restriction enzymes and inserted into the C terminal region of GFP ORF of the pEGFC1 vector (Clontech). Using Tfx-50 (Promega), this plasmid that encodes the GFP-Tesmin fusion protein was introduced into COS1 cells growing on a cover glass. The cover glass was fixed by methanol/acetone (1:1) and washed three times with PBS. The cells were observed with Olympus BH-2 Epifluorescent Microscope. As a result, although the protein fused to the full sequence of Tesmin was localized within the cytoplasm, one having the partially deleted Tesmin sequence had migrated into the nucleus (Fig. 8).

Example 11: Preparation of a specific antibody that binds to the Tesmin protein

A peptide antibody against the 18 amino acids presumed by the gene arrangement of Tesmin was prepared. Specifically, an 18 amino acid sequence (SEQ ID NO: 22) was made using a peptide synthesizer. KLH was covalently bound to this obtained peptide with a crosslinking reagent. Next, this peptide was purified by HPLC, and a rabbit was immunized with it. Serum was drawn out at four stages, and finally, all the blood was collected. This serum was purified using a protein A column to prepare the polyclonal antibody. Tesmin protein fused with GST was separated on a gel by SDS-PAGE. Detection by Western blotting confirmed that this anti Tesmin polyclonal antibody recognizes the Tesmin protein (Fig.

9).

Western blotting was done by inducing recombinant protein expression through isopropyl-β-D-thiogalactoside (IPTG) added to Tesmin-cDNA-introduced *E. coli*, and subjecting an *E. coli* lysate to SDS-PAGE (IPTG+, Fig. 9). A detection using a cell lysate of *E. coli* without IPTG was also done (IPTG-, Fig. 9).

Industrial Applicability

The present invention provides the Tesmin protein comprising a metal-binding site, which is closely associated with the differentiation of testicular cells, and the gene thereof. The Tesmin protein and the gene thereof are involved in the differentiation during spermatogenesis, and Tesmin gene expression is not seen in infertile mice. Therefore, this gene may also be the causative gene of the genetic disease of infertile mice. Hence, it is anticip ated that gene therapy of infertility would be possible by introducing the Tesmin gene into the body or cells. Moreover, Tesmin is expressed in the testis only, and even in the testis, the expression is seen only at limited stages. Therefore, Tesmin may also be applied as a test drug for determining the differentiation stage of testicular cells. Tesmin is also thought to contain a metal-binding site similar to metallothionein, and therefore, can also be applied as a metal-poison neutralizing agent similar to metallothionein. It is also expected to be utilized in applied studies such as those analyzing the importance of metal binding in the testis.

CLAIMS

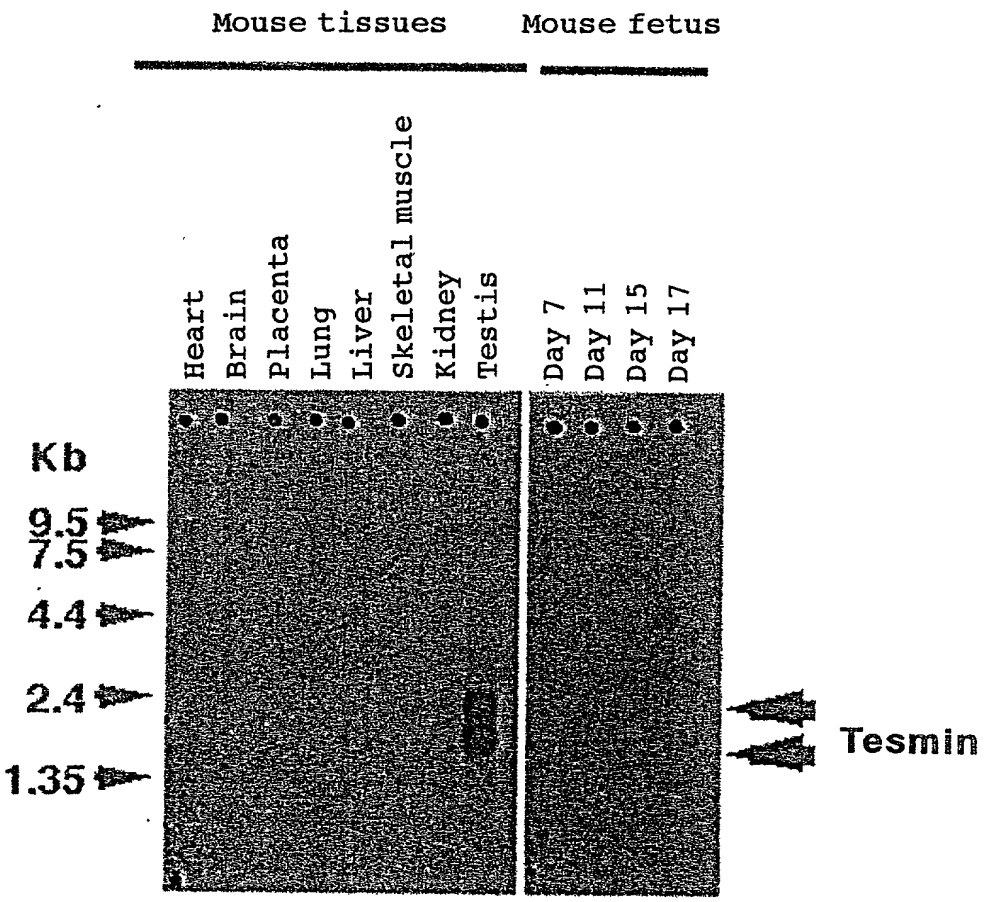
1. A protein comprising the amino acid sequence of SEQ ID NO: 4 or 5.
2. A protein which comprises an amino acid sequence in which one or several amino acids in the amino acid sequence of SEQ ID NO: 4 or 5 have been replaced, deleted, and/or added, and which is functionally equivalent to the protein of claim 1.
3. A protein which is encoded by a DNA hybridizing to the DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3, and which is functionally equivalent to the protein of claim 1.
4. A DNA encoding the protein of any one of claims 1 to 3.
5. A vector comprising the DNA of claim 4.
6. A transformant comprising the DNA of claim 4 in an expressible manner.
7. A method of producing the protein of any one of claims 1 to 3 comprising the steps of culturing the transformant of claim 6, and collecting the expressed protein from said transformant or the culture supernatant thereof.
8. An antibody binding to the protein of any one of claims 1 to 3.
9. A DNA specifically hybridizing to a DNA comprising the nucleotide sequence of any one of SEQ ID NOs: 1 to 3, and comprising at least 15 nucleotides.

ABSTRACT

A gene expressed specifically in the testis has been unexpectedly isolated in the course of studies of the expression of a gene encoding an unknown protein that triggers cell death. The isolated gene was a novel gene sequence that had no significant homologue in the database. This gene was also found to be involved in the regulation of differentiation in the testis.

09/743237

Figure 1



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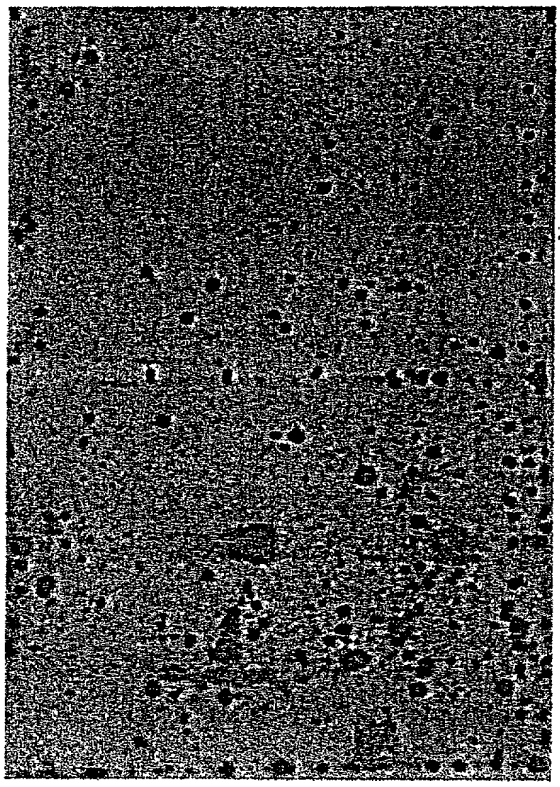
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Colon
Leukocyte

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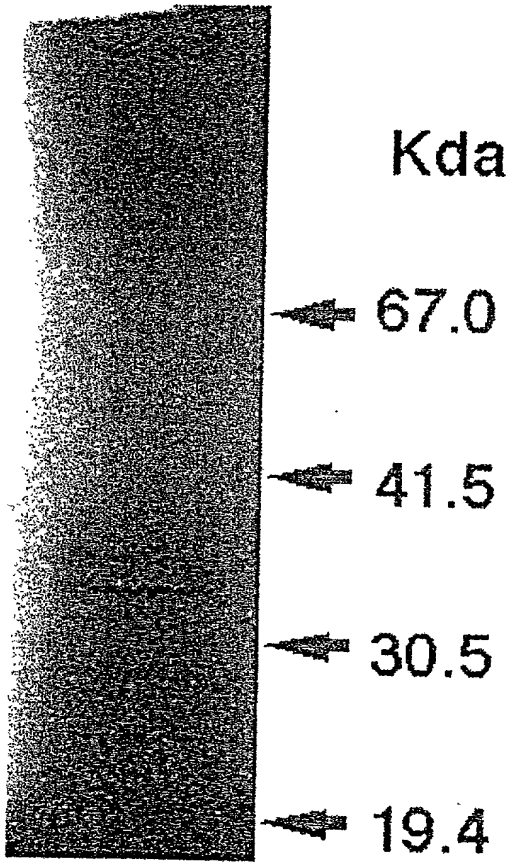

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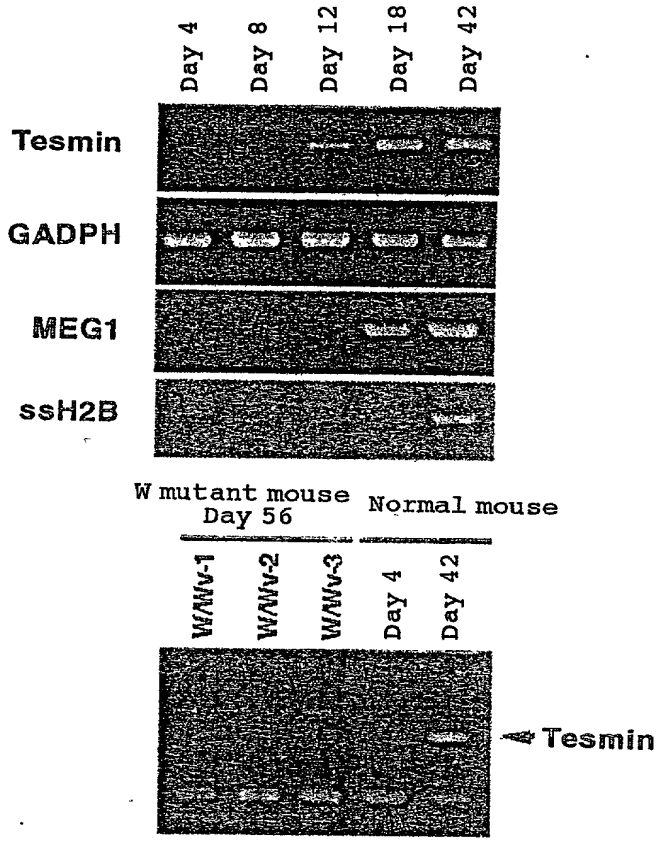

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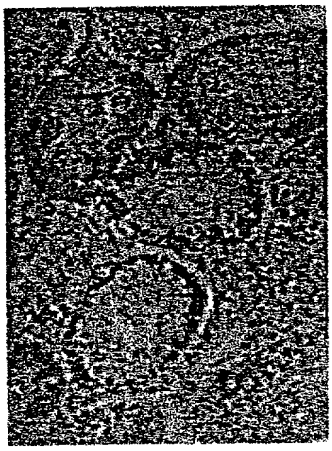

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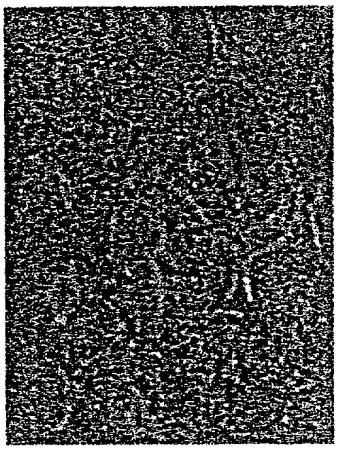
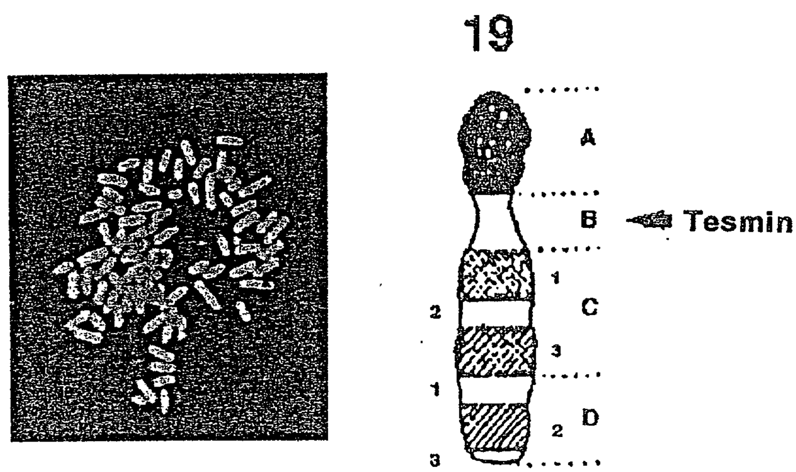


Tesmin-sense primer

Figure 6



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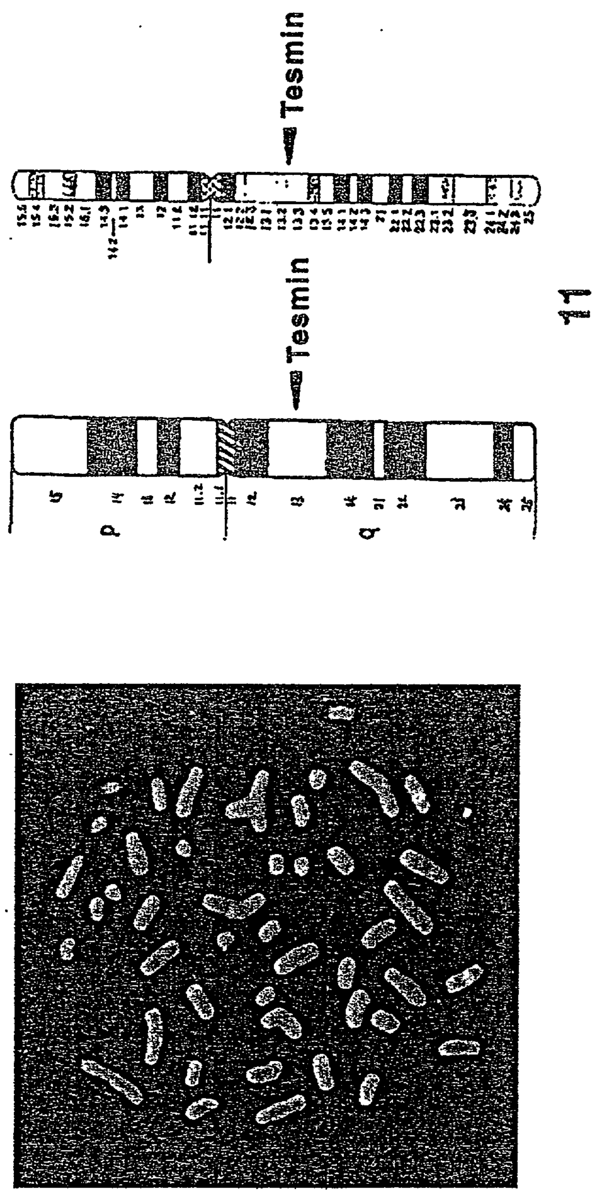

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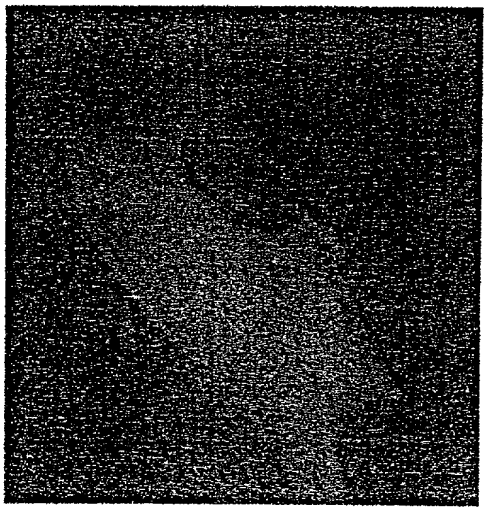
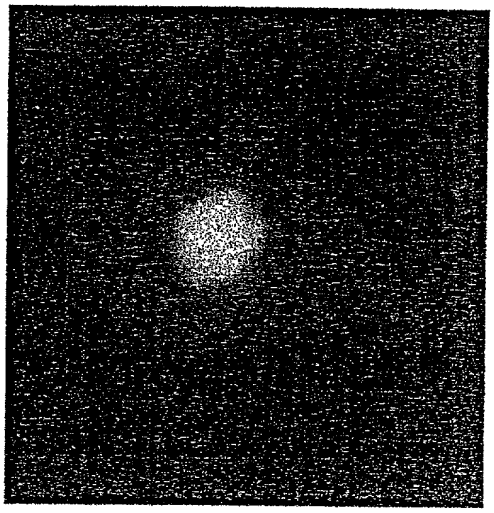
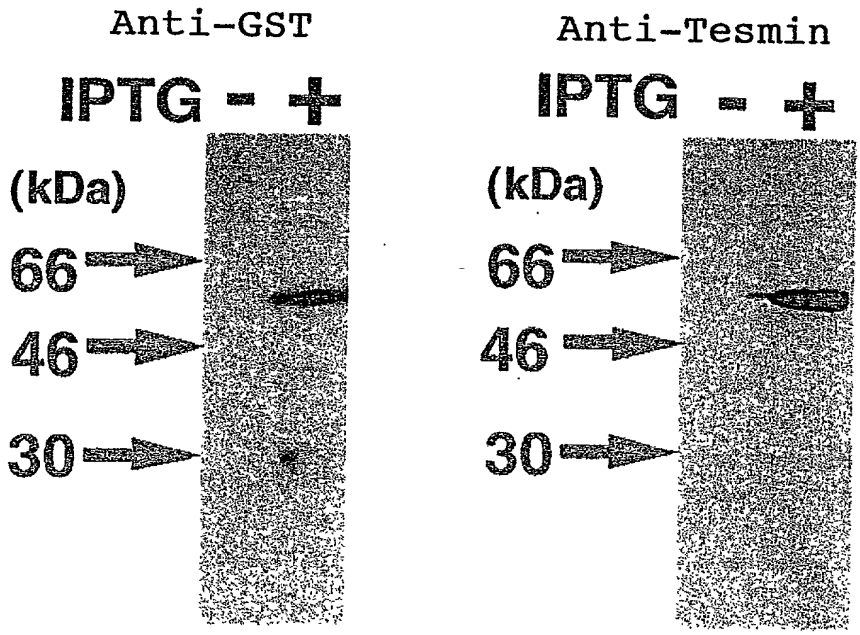


pEGFC1-complete Tesmin



pEGFC1-Tesmin deletant

Figure 9



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 084335/0127

In re patent application of:
Takashi Sugihara et al.

Serial No.: 09/743,237

Filed: January 5, 2001

Entitled: TESTIS-SPECIFIC DIFFERENTIATION-REGULATORY FACTOR

ASSOCIATE POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The undersigned attorney of record hereby appoints Stephen B. Maebius, Registration No. 35,264 as an associate attorney with full power of association, substitution and revocation, to prosecute the above-identified application and transact all business in the Patent and Trademark Office connected therewith.

Please address all correspondence to: STEPHEN B. MAEBIUS

Respectfully submitted,

June 4, 2001
Date

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DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name;

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

TESTIS-SPECIFIC DIFFERENTIATION-REGULATORY FACTOR

(Attorney Docket No. 084335/0127)

the specification of which (check one)

___ is attached hereto.

__X__ was filed on July 16, 1999 as United States Application Number or PCT International Application Number PCT/JP99/03859 and was amended on _____ (if applicable).

THAT I do not know and do not believe that the same invention was ever known or used by others in the United States of America, or was patented or described in any printed publication in any country, before I (we) invented it;

THAT I do not know and do not believe that the same invention was patented or described in any printed publication in any country, or in public use or on sale in the United States of America, for more than one year prior to the filing date of this United States application;

THAT I do not know and do not believe that the same invention was first patented or made the subject of an inventor's certificate that issued in any country foreign to the United States of America before the filing date of this United States application if the foreign application was filed by me (us), or by my (our) legal representatives or assigns, more than twelve months (six months for design patents) prior to the filing date of this United States application;

THAT I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment specifically referred to above;

THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

THAT I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I HEREBY CLAIM foreign priority benefits under Title 35, United States Code §119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number	Country	Foreign Filing Date	Priority Claimed?	Certified Copy Attached?
10-219856	Japan	July 17, 1998	Yes	
PCT/JP99/03859	PCT	July 16, 1999	Yes	

I HEREBY CLAIM the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

U.S. Provisional Application Number	Filing Date

I HEREBY CLAIM the benefit under Title 35, United States Code, §120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Application Number	Parent Filing Date	Parent Patent Number

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to have full power to prosecute this application and any continuations, divisions, reissues, and reexaminations thereof, to receive the patent, and to transact all business in the United States Patent and Trademark Office connected therewith.

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I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Inventor's signature	Youji Mitsui
Date	17th April 2001

SEQUENCE LISTING

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SECRETARY OF AGENCY OF INDUSTRIAL SCIENCE AND TECHNOLOGY

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Ile Cys Gln Leu Lys Gly Gly Ala Gln Met Leu Cys Ile Asp Asn Cys
5 10 15

ggc gcg agg gag ctc aaa gcg ctc cat ctg ctt cct cag tac gat gac 752
Gly Ala Arg Glu Leu Lys Ala Leu His Leu Leu Pro Gln Tyr Asp Asp
20 25 30

cag agc agt ttc cct cag tca gag ctc cct aag cca atg aca act tta 800
Gln Ser Ser Phe Pro Gln Ser Glu Leu Pro Lys Pro Met Thr Thr Leu
35 40 45 50

gtg gga aga ctt ctg cca gta cca gcg aag tta aat ctc atc aca cag 848
Val Gly Arg Leu Leu Pro Val Pro Ala Lys Leu Asn Leu Ile Thr Gln
55 60 65

gtt gat aat gga gct ctc cca tca gct gtc aat ggg gct gcc ttt ccc 896
Val Asp Asn Gly Ala Leu Pro Ser Ala Val Asn Gly Ala Ala Phe Pro
70 75 80

tct gga cct gct ctg caa ggg cca ccc aaa ata act ctg tct ggg tac 944
Ser Gly Pro Ala Leu Gln Gly Pro Pro Lys Ile Thr Leu Ser Gly Tyr
85 90 95

tgt gac tgc ttc tcc agc ggg gac ttc tgc aac agc tgc agc tgc aac 992
Cys Asp Cys Phe Ser Ser Gly Asp Phe Cys Asn Ser Cys Ser Cys Asn
100 105 110

aac ctg cgc cat gag ctc gag cgc ttc aaa gcc ata aag gcg tgt ctt 1040
Asn Leu Arg His Glu Leu Glu Arg Phe Lys Ala Ile Lys Ala Cys Leu
115 120 125 130

gat aga aat cct gaa gct ttc caa cca aaa atg ggg aaa ggc cgt ctg 1088
Asp Arg Asn Pro Glu Ala Phe Gln Pro Lys Met Gly Lys Gly Arg Leu
135 140 145

gga gct gct aaa ctt cga cac agc aaa ggg tgc aac tgt aag cgc tca 1136
Gly Ala Ala Lys Leu Arg His Ser Lys Gly Cys Asn Cys Lys Arg Ser
150 155 160

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Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr Glu Ala Lys Ile Met Cys
165 170 175

tct tcc att tgc aaa tgc att gct tgc aaa aac tat gaa gaa agt cca 1232
Ser Ser Ile Cys Lys Cys Ile Ala Cys Lys Asn Tyr Glu Glu Ser Pro
180 185 190

gaa cga aaa atg ctg atg agc aca ccc cac tac atg gag cct ggg gac 1280
Glu Arg Lys Met Leu Met Ser Thr Pro His Tyr Met Glu Pro Gly Asp
195 200 205 210

ttt gag agc agc cat tat ttg tcc cca gcc aag ttc tca gga cct cca 1328
Phe Glu Ser Ser His Tyr Leu Ser Pro Ala Lys Phe Ser Gly Pro Pro
215 220 225

aaa ctg aga aaa aat agg cag gcc ttc tcc tgt atc tcc tgg gaa gta 1376
Lys Leu Arg Lys Asn Arg Gln Ala Phe Ser Cys Ile Ser Trp Glu Val
230 235 240

gtg gag gcc aca tgt gcc tgc ctg ctg gcc cag ggt gag gaa gca gag 1424
Val Glu Ala Thr Cys Ala Cys Leu Leu Ala Gln Gly Glu Glu Ala Glu
245 250 255

cag gag cac tgt tcc cca agc ttg gct gag cag atg atc ctg gag gag 1472
Gln Glu His Cys Ser Pro Ser Leu Ala Glu Gln Met Ile Leu Glu Glu
260 265 270

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275 280 285 290

ggg ctg aaa att gag tagcgtgcaa gctggtaaag gggaatgcct gtggcaagcc 1575
Gly Leu Lys Ile Glu
295

tcagccctgg gaatctgcac cgaggaagct ggtgcccagg gaggagcaga ggccgcgcat 1635

catggccagg tcagctgtga ggtctgagtg atctgcatgg tactggccag cctactcaag 1695

gtatcctaaa gtgcaagcag gcagagccac cctggggatg gacactggcc ctcctgtccc 1755

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gttatgtgtt tgctttcaaa ttgcttagta gtacctccat tcaagttatt atgagccagc 1875

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ggttagagct gggtttgttt ggattttttg cgttttttc ttcctgtatt tctgcttgaa 2055

gtgagaaaac ttgtctcctg tccaaccttt tctccataat tactgctgca cggtcgcctg 2115

ctgaccagtc acagtgacct cagacaccag aaggtgaggt ggcttattat gcccacactt 2175

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atcaaagaag caggtggtag tgtgccaggc ggcagccctg aagacgcagc tttccaggcc 180

cctctggctc aggaatcctg ttgcaagttc ccatcatccc aggaggcaga ggaggcctcc 240

agctgccctc ggaagaaaga ctccagcccc atg gtg att tgt cag ctg aaa gga 294
Met Val Ile Cys Gln Leu Lys Gly
1 5

ggc gcc cag atg ctc tgc ata gac aac tgt ggc gcg agg gag ctc aaa 342
Gly Ala Gln Met Leu Cys Ile Asp Asn Cys Gly Ala Arg Glu Leu Lys
10 15 20

gcg ctc cat ctg ctt cct cag tac gat gac cag agc agt ttc cct cag 390
Ala Leu His Leu Leu Pro Gln Tyr Asp Asp Gln Ser Ser Phe Pro Gln

25 30 35 40

tca gag ctc cct aag cca atg aca act tta gtg gga aga ctt ctg cca 438
Ser Glu Leu Pro Lys Pro Met Thr Thr Leu Val Gly Arg Leu Leu Pro
45 50 55

gta cca gcg aag tta aat ctc atc aca cag gtt gat aat gga gct ctc 486
Val Pro Ala Lys Leu Asn Leu Ile Thr Gln Val Asp Asn Gly Ala Leu
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Pro Ser Ala Val Asn Gly Ala Ala Phe Pro Ser Gly Pro Ala Leu Gln
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90 95 100

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105 110 115 120

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125 130 135

ttc caa cca aaa atg ggg aaa ggc cgt ctg gga gct gct aaa ctt cga 726
Phe Gln Pro Lys Met Gly Lys Gly Arg Leu Gly Ala Ala Lys Leu Arg
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cac agc aaa ggg tgc aac tgt aag cgc tca ggc tgc ctg aag aac tac 774
His Ser Lys Gly Cys Asn Cys Lys Arg Ser Gly Cys Leu Lys Asn Tyr
155 160 165

tgt gag tgc tat gag gcc aaa atc atg tgt tct tcc att tgc aaa tgc 822
Cys Glu Cys Tyr Glu Ala Lys Ile Met Cys Ser Ser Ile Cys Lys Cys

170 175 180

att gct tgc aaa aac tat gaa gaa agt cca gaa cga aaa atg ctg atg 870
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agc aca ccc cac tac atg gag cct ggg gac ttt gag agc agc cat tat 918
Ser Thr Pro His Tyr Met Glu Pro Gly Asp Phe Glu Ser Ser His Tyr
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cag gcc ttc tcc tgt atc tcc tgg gaa gta gtg gag gcc aca tgt gcc 1014
Gln Ala Phe Ser Cys Ile Ser Trp Glu Val Val Glu Ala Thr Cys Ala
235 240 245

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Cys Leu Leu Ala Gln Gly Glu Glu Ala Glu Gln Glu His Cys Ser Pro
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agc ttg gct gag cag atg atc ctg gag gag ttt gga agg tgc ctg tcg 1110
Ser Leu Ala Glu Gln Met Ile Leu Glu Glu Phe Gly Arg Cys Leu Ser
265 270 275 280

cag att ctc cac atc gag ttc aag tcc aag ggg ctg aaa att gag 1155
Gln Ile Leu His Ile Glu Phe Lys Ser Lys Gly Leu Lys Ile Glu
285 290 295

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aacctttcca gactcccaaa aaaaaaaaaa aaaaaaaaaa aaaaaa 1861

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gggtgttttg cccctggggc gcctgggtcc tgcgaaggag cctcccaccc gggcgtccgc 240

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agcaactttg cagaatcttc ttgctcagga atcctgttgc aagttcccat ggtcccagga 360

actagaggat gcctcctgct gttctcttaa gaaagattcc aaccca atg gtg ata 415
Met Val Ile
1

tgc caa ttg aaa ggg ggc aca caa atg cta tgt ata gac aat tct aga 463
Cys Gln Leu Lys Gly Gly Thr Gln Met Leu Cys Ile Asp Asn Ser Arg
5 10 15

aca aga gaa cta aaa gca ctc cat ttg gtt cct cag tat caa gat caa 511
Thr Arg Glu Leu Lys Ala Leu His Leu Val Pro Gln Tyr Gln Asp Gln
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aat aat tat cta cag tca gat gtc cct aaa cca atg act gct tta gta 559
Asn Asn Tyr Leu Gln Ser Asp Val Pro Lys Pro Met Thr Ala Leu Val
40 45 50

ggg aga ttt ttg cca gca tca aca aaa tta aat ctc att aca caa caa 607
Gly Arg Phe Leu Pro Ala Ser Thr Lys Leu Asn Leu Ile Thr Gln Gln
55 60 65

ctt gag gga gcc tta cca tcg gta gtc aac ggg tct gct ttc ccc tcg 655
Leu Glu Gly Ala Leu Pro Ser Val Val Asn Gly Ser Ala Phe Pro Ser
70 75 80

gga tca act ctt cca gga cca cca aaa ata act ttg gct ggg tac tgt 703
Gly Ser Thr Leu Pro Gly Pro Pro Lys Ile Thr Leu Ala Gly Tyr Cys
85 90 95

gac tgc ttt gcc agt ggg gac ttt tgc aac aac tgc aat tgt aat aat 751
Asp Cys Phe Ala Ser Gly Asp Phe Cys Asn Asn Cys Asn Cys Asn Asn
100 105 110 115

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tgt tgc aac aac ttg cat cat gat att gaa cgg ttt aaa gcc att aag   799
Cys Cys Asn Asn Leu His His Asp Ile Glu Arg Phe Lys Ala Ile Lys
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gca tgt ctt ggt aga aat cca gaa gct ttc cag cca aaa att ggg aag   847
Ala Cys Leu Gly Arg Asn Pro Glu Ala Phe Gln Pro Lys Ile Gly Lys
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ggc caa ttg ggc aat gtc aag ccc cag cac aac aaa ggg tgc aac tgc   895
Gly Gln Leu Gly Asn Val Lys Pro Gln His Asn Lys Gly Cys Asn Cys
        150                 155                 160

agg agg tca ggc tgc ctg aag aat tac tgc gag tgc tat gag gcc caa   943
Arg Arg Ser Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr Glu Ala Gln
    165                 170                 175

att atg tgt tct tct att tgc aaa tgc att ggt tgc aaa aat tat gaa   991
Ile Met Cys Ser Ser Ile Cys Lys Cys Ile Gly Cys Lys Asn Tyr Glu
180                 185                 190                 195

gaa agc cca gaa cga aag aca cta atg agc atg cca aac tac atg cag   1039
Glu Ser Pro Glu Arg Lys Thr Leu Met Ser Met Pro Asn Tyr Met Gln
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act gga ggt ttg gaa ggc agc cat tac ctg cca cca acg aaa ttt tca   1087
Thr Gly Gly Leu Glu Gly Ser His Tyr Leu Pro Pro Thr Lys Phe Ser
            215                 220                 225

gga ctt cca aga ttc agt cac gat agg cgg cct tcc tca tgc atc tcc   1135
Gly Leu Pro Arg Phe Ser His Asp Arg Arg Pro Ser Ser Cys Ile Ser
        230                 235                 240

tgg gag gtg gtg gag gcc aca tgc gcc tgc ctg ctt gct cag gga gaa   1183
Trp Glu Val Val Glu Ala Thr Cys Ala Cys Leu Leu Ala Gln Gly Glu
    245                 250                 255
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gag gcc gag aaa gaa cac tgc tcc aag tgc ctg gca gag cag atg atc 1231
Glu Ala Glu Lys Glu His Cys Ser Lys Cys Leu Ala Glu Gln Met Ile
260 265 270 275

ctg gag gaa ttt gga agg tgc tta tca cag att ctc cac act gag ttt 1279
Leu Glu Glu Phe Gly Arg Cys Leu Ser Gln Ile Leu His Thr Glu Phe
280 285 290

aaa tct aag gga ttg aaa atg gag tagagtataa agtgtgaatg catgttgatt 1333
Lys Ser Lys Gly Leu Lys Met Glu
295

ttgtcttagt ctagaaatct ctagtttaga aaggatgttt aggggaacat gaggctggct 1393

ctgcagcaac aaccaggctc ccctgcatcc ctgggcccag ggagtttact cagagctctc 1453

tgaagatgtg gcaacccatg ccccctttc tgaggaggtg catggcctga gcattgtttg 1513

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ctgaacacct aaagattttc tgtagcgtag atatttcccc agagacacgc gaactgtcag 1813

tctttcctaa ggccccggg agacgcaggc aatggggcct cgcaggccag gcttgcacca 1873

gcatgtcttg agttagagga cttaaaatta tccagtttct tctgtgtttc tacttgaatt 1933

gtggaaaagc tctattatcc aattaacttc tccataatta ttgttgtaat attattattg 1993

tttgtaaaac atggttcaca taactagctt gtggaaacca gcaggtaaaa tgaattctta 2053

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Asp Asp Gln Ser Ser Phe Pro Gln Ser Glu Leu Pro Lys Pro Met Thr
35 40 45

Thr Leu Val Gly Arg Leu Leu Pro Val Pro Ala Lys Leu Asn Leu Ile
50 55 60

Thr Gln Val Asp Asn Gly Ala Leu Pro Ser Ala Val Asn Gly Ala Ala
65 70 75 80

Phe Pro Ser Gly Pro Ala Leu Gln Gly Pro Pro Lys Ile Thr Leu Ser
85 90 95

Gly Tyr Cys Asp Cys Phe Ser Ser Gly Asp Phe Cys Asn Ser Cys Ser
100 105 110

Cys Asn Asn Leu Arg His Glu Leu Glu Arg Phe Lys Ala Ile Lys Ala
115 120 125

Cys Leu Asp Arg Asn Pro Glu Ala Phe Gln Pro Lys Met Gly Lys Gly
130 135 140

Arg Leu Gly Ala Ala Lys Leu Arg His Ser Lys Gly Cys Asn Cys Lys
145 150 155 160

Arg Ser Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr Glu Ala Lys Ile
165 170 175

Met Cys Ser Ser Ile Cys Lys Cys Ile Ala Cys Lys Asn Tyr Glu Glu
180 185 190

Ser Pro Glu Arg Lys Met Leu Met Ser Thr Pro His Tyr Met Glu Pro
195 200 205

Gly Asp Phe Glu Ser Ser His Tyr Leu Ser Pro Ala Lys Phe Ser Gly
210 215 220

Pro Pro Lys Leu Arg Lys Asn Arg Gln Ala Phe Ser Cys Ile Ser Trp
225 230 235 240

Glu Val Val Glu Ala Thr Cys Ala Cys Leu Leu Ala Gln Gly Glu Glu
245 250 255

Ala Glu Gln Glu His Cys Ser Pro Ser Leu Ala Glu Gln Met Ile Leu
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Ser Lys Gly Leu Lys Ile Glu
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Gln Asp Gln Asn Asn Tyr Leu Gln Ser Asp Val Pro Lys Pro Met Thr
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Ala Leu Val Gly Arg Phe Leu Pro Ala Ser Thr Lys Leu Asn Leu Ile
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Thr Gln Gln Leu Glu Gly Ala Leu Pro Ser Val Val Asn Gly Ser Ala
65                  70                  75                  80

Phe Pro Ser Gly Ser Thr Leu Pro Gly Pro Pro Lys Ile Thr Leu Ala
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Gly Tyr Cys Asp Cys Phe Ala Ser Gly Asp Phe Cys Asn Asn Cys Asn
            100                 105                 110

Cys Asn Asn Cys Cys Asn Asn Leu His His Asp Ile Glu Arg Phe Lys
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Ala Ile Lys Ala Cys Leu Gly Arg Asn Pro Glu Ala Phe Gln Pro Lys
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Ile Gly Lys Gly Gln Leu Gly Asn Val Lys Pro Gln His Asn Lys Gly
145                 150                 155                 160

Cys Asn Cys Arg Arg Ser Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr
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Glu Ala Gln Ile Met Cys Ser Ser Ile Cys Lys Cys Ile Gly Cys Lys
            180                 185                 190
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Asn Tyr Glu Glu Ser Pro Glu Arg Lys Thr Leu Met Ser Met Pro Asn
195 200 205

Tyr Met Gln Thr Gly Gly Leu Glu Gly Ser His Tyr Leu Pro Pro Thr
210 215 220

Lys Phe Ser Gly Leu Pro Arg Phe Ser His Asp Arg Arg Pro Ser Ser
225 230 235 240

Cys Ile Ser Trp Glu Val Val Glu Ala Thr Cys Ala Cys Leu Leu Ala
245 250 255

Gln Gly Glu Glu Ala Glu Lys Glu His Cys Ser Lys Cys Leu Ala Glu
260 265 270

Gln Met Ile Leu Glu Glu Phe Gly Arg Cys Leu Ser Gln Ile Leu His
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Thr Glu Phe Lys Ser Lys Gly Leu Lys Met Glu
290 295

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Ile Glu

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<110> SUGIHARA, TAKASHI
WADHWA, RENU
KAUL, SUNIL C.
MITSUI, YOUJI

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ggggttggct gggagatttt tttttcttca caccaaagac ttccattatt gaggattttt 240

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tatataactt aggagaacct ctgattctcc tgcctctaca tcctgagtgc taggtgtaca 420

gggggaaatc attttggtga gactccgatg aactactgcc aggttcccaa ggcagcaagc 480

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aagacgcagc tttccaggcc cctctggctc aggaatcctg ttgcaagttc ccatcatccc 600

aggaggcaga ggaggcctcc agctgccctc ggaagaaaga ctccagcccc atg gtg 656
Met Val
1

att tgt cag ctg aaa gga ggc gcc cag atg ctc tgc ata gac aac tgt 704
Ile Cys Gln Leu Lys Gly Gly Ala Gln Met Leu Cys Ile Asp Asn Cys
5 10 15

```
ggc gcg agg gag ctc aaa gcg ctc cat ctg ctt cct cag tac gat gac   752
Gly Ala Arg Glu Leu Lys Ala Leu His Leu Leu Pro Gln Tyr Asp Asp
     20                  25                  30

cag agc agt ttc cct cag tca gag ctc cct aag cca atg aca act tta   800
Gln Ser Ser Phe Pro Gln Ser Glu Leu Pro Lys Pro Met Thr Thr Leu
 35                  40                  45                  50

gtg gga aga ctt ctg cca gta cca gcg aag tta aat ctc atc aca cag   848
Val Gly Arg Leu Leu Pro Val Pro Ala Lys Leu Asn Leu Ile Thr Gln
                 55                  60                  65

gtt gat aat gga gct ctc cca tca gct gtc aat ggg gct gcc ttt ccc   896
Val Asp Asn Gly Ala Leu Pro Ser Ala Val Asn Gly Ala Ala Phe Pro
             70                  75                  80

tct gga cct gct ctg caa ggg cca ccc aaa ata act ctg tct ggg tac   944
Ser Gly Pro Ala Leu Gln Gly Pro Pro Lys Ile Thr Leu Ser Gly Tyr
         85                  90                  95

tgt gac tgc ttc tcc agc ggg gac ttc tgc aac agc tgc agc tgc aac   992
Cys Asp Cys Phe Ser Ser Gly Asp Phe Cys Asn Ser Cys Ser Cys Asn
    100                 105                 110

aac ctg cgc cat gag ctc gag cgc ttc aaa gcc ata aag gcg tgt ctt  1040
Asn Leu Arg His Glu Leu Glu Arg Phe Lys Ala Ile Lys Ala Cys Leu
115                 120                 125                 130

gat aga aat cct gaa gct ttc caa cca aaa atg ggg aaa ggc cgt ctg  1088
Asp Arg Asn Pro Glu Ala Phe Gln Pro Lys Met Gly Lys Gly Arg Leu
                135                 140                 145

gga gct gct aaa ctt cga cac agc aaa ggg tgc aac tgt aag cgc tca  1136
Gly Ala Ala Lys Leu Arg His Ser Lys Gly Cys Asn Cys Lys Arg Ser
            150                 155                 160

ggc tgc ctg aag aac tac tgt gag tgc tat gag gcc aaa atc atg tgt  1184
Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr Glu Ala Lys Ile Met Cys
        165                 170                 175

tct tcc att tgc aaa tgc att gct tgc aaa aac tat gaa gaa agt cca  1232
Ser Ser Ile Cys Lys Cys Ile Ala Cys Lys Asn Tyr Glu Glu Ser Pro
    180                 185                 190

gaa cga aaa atg ctg atg agc aca ccc cac tac atg gag cct ggg gac  1280
Glu Arg Lys Met Leu Met Ser Thr Pro His Tyr Met Glu Pro Gly Asp
195                 200                 205                 210

ttt gag agc agc cat tat ttg tcc cca gcc aag ttc tca gga cct cca  1328
Phe Glu Ser Ser His Tyr Leu Ser Pro Ala Lys Phe Ser Gly Pro Pro
                215                 220                 225

aaa ctg aga aaa aat agg cag gcc ttc tcc tgt atc tcc tgg gaa gta  1376
Lys Leu Arg Lys Asn Arg Gln Ala Phe Ser Cys Ile Ser Trp Glu Val
            230                 235                 240
```

```
gtg gag gcc aca tgt gcc tgc ctg ctg gcc cag ggt gag gaa gca gag   1424
Val Glu Ala Thr Cys Ala Cys Leu Leu Ala Gln Gly Glu Glu Ala Glu
        245                 250                 255

cag gag cac tgt tcc cca agc ttg gct gag cag atg atc ctg gag gag   1472
Gln Glu His Cys Ser Pro Ser Leu Ala Glu Gln Met Ile Leu Glu Glu
    260                 265                 270

ttt gga agg tgc ctg tcg cag att ctc cac atc gag ttc aag tcc aag   1520
Phe Gly Arg Cys Leu Ser Gln Ile Leu His Ile Glu Phe Lys Ser Lys
275                 280                 285                 290

ggg ctg aaa att gag tagcgtgcaa gctggtaaag gggaatgcct gtggcaagcc   1575
Gly Leu Lys Ile Glu
               295

tcagccctgg gaatctgcac cgaggaagct ggtgcccagg gaggagcaga ggccgcgcat 1635

catggccagg tcagctgtga ggtctgagtg atctgcatgg tactggccag cctactcaag 1695

gtatcctaaa gtgcaagcag gcagagccac cctggggatg gacactggcc ctcctgtccc 1755

tggggaggcc ctctggggac tccctgccct gcataaaaag agggtgattt tctacttgtt 1815

gttatgtgtt tgctttcaaa ttgcttagta gtacctccat tcaagttatt atgagccagc 1875

ctcaagttag agagctaggc tcttcttcag gtggactctg cccaaatcac atacaagtca 1935

ggtggccatc aggggttttt ccaggccagg cctgtgacag gagatatggg agggggggtcg 1995

ggttagagct gggtttgttt ggatttttg cgttttttc ttcctgtatt tctgcttgaa 2055

gtgagaaaac ttgtctcctg tccaaccttt tctccataat tactgctgca cggtcgcctg 2115

ctgaccagtc acagtgacct cagacaccag aaggtgaggt ggcttattat gcccacactt 2175

tgtgttttgt tgtgagaata aacctttcca gactcccaaa aaaaaaaaaa aaaaaaaaaa 2235

aaaaaa                                                            2241
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<210> 2
<211> 1861
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (271)..(1155)

<400> 2

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ggtgctcgcg ggcccgcgct gttgccgcta agcgcaggag tgcgcgtgat cccagttgaa 120

atcaaagaag caggtggtag tgtgccaggc ggcagccctg aagacgcagc tttccaggcc 180

cctctggctc aggaatcctg ttgcaagttc ccatcatccc aggaggcaga ggaggcctcc 240
```

```
agctgccctc ggaagaaaga ctccagcccc atg gtg att tgt cag ctg aaa gga  294
                                  Met Val Ile Cys Gln Leu Lys Gly
                                    1               5

ggc gcc cag atg ctc tgc ata gac aac tgt ggc gcg agg gag ctc aaa   342
Gly Ala Gln Met Leu Cys Ile Asp Asn Cys Gly Ala Arg Glu Leu Lys
     10                  15                  20

gcg ctc cat ctg ctt cct cag tac gat gac cag agc agt ttc cct cag   390
Ala Leu His Leu Leu Pro Gln Tyr Asp Asp Gln Ser Ser Phe Pro Gln
 25                  30                  35                  40

tca gag ctc cct aag cca atg aca act tta gtg gga aga ctt ctg cca   438
Ser Glu Leu Pro Lys Pro Met Thr Thr Leu Val Gly Arg Leu Leu Pro
                 45                  50                  55

gta cca gcg aag tta aat ctc atc aca cag gtt gat aat gga gct ctc   486
Val Pro Ala Lys Leu Asn Leu Ile Thr Gln Val Asp Asn Gly Ala Leu
             60                  65                  70

cca tca gct gtc aat ggg gct gcc ttt ccc tct gga cct gct ctg caa   534
Pro Ser Ala Val Asn Gly Ala Ala Phe Pro Ser Gly Pro Ala Leu Gln
         75                  80                  85

ggg cca ccc aaa ata act ctg tct ggg tac tgt gac tgc ttc tcc agc   582
Gly Pro Pro Lys Ile Thr Leu Ser Gly Tyr Cys Asp Cys Phe Ser Ser
     90                  95                  100

ggg gac ttc tgc aac agc tgc agc tgc aac aac ctg cgc cat gag ctc   630
Gly Asp Phe Cys Asn Ser Cys Ser Cys Asn Asn Leu Arg His Glu Leu
105                 110                 115                 120

gag cgc ttc aaa gcc ata aag gcg tgt ctt gat aga aat cct gaa gct   678
Glu Arg Phe Lys Ala Ile Lys Ala Cys Leu Asp Arg Asn Pro Glu Ala
                125                 130                 135

ttc caa cca aaa atg ggg aaa ggc cgt ctg gga gct gct aaa ctt cga   726
Phe Gln Pro Lys Met Gly Lys Gly Arg Leu Gly Ala Ala Lys Leu Arg
            140                 145                 150

cac agc aaa ggg tgc aac tgt aag cgc tca ggc tgc ctg aag aac tac   774
His Ser Lys Gly Cys Asn Cys Lys Arg Ser Gly Cys Leu Lys Asn Tyr
        155                 160                 165

tgt gag tgc tat gag gcc aaa atc atg tgt tct tcc att tgc aaa tgc   822
Cys Glu Cys Tyr Glu Ala Lys Ile Met Cys Ser Ser Ile Cys Lys Cys
    170                 175                 180

att gct tgc aaa aac tat gaa gaa agt cca gaa cga aaa atg ctg atg   870
Ile Ala Cys Lys Asn Tyr Glu Glu Ser Pro Glu Arg Lys Met Leu Met
185                 190                 195                 200

agc aca ccc cac tac atg gag cct ggg gac ttt gag agc agc cat tat   918
Ser Thr Pro His Tyr Met Glu Pro Gly Asp Phe Glu Ser Ser His Tyr
                205                 210                 215
```

```
ttg tcc cca gcc aag ttc tca gga cct cca aaa ctg aga aaa aat agg   966
Leu Ser Pro Ala Lys Phe Ser Gly Pro Pro Lys Leu Arg Lys Asn Arg
            220                 225                 230

cag gcc ttc tcc tgt atc tcc tgg gaa gta gtg gag gcc aca tgt gcc   1014
Gln Ala Phe Ser Cys Ile Ser Trp Glu Val Val Glu Ala Thr Cys Ala
        235                 240                 245

tgc ctg ctg gcc cag ggt gag gaa gca gag cag gag cac tgt tcc cca   1062
Cys Leu Leu Ala Gln Gly Glu Glu Ala Glu Gln Glu His Cys Ser Pro
    250                 255                 260

agc ttg gct gag cag atg atc ctg gag gag ttt gga agg tgc ctg tcg   1110
Ser Leu Ala Glu Gln Met Ile Leu Glu Glu Phe Gly Arg Cys Leu Ser
265                 270                 275                 280

cag att ctc cac atc gag ttc aag tcc aag ggg ctg aaa att gag       1155
Gln Ile Leu His Ile Glu Phe Lys Ser Lys Gly Leu Lys Ile Glu
                285                 290                 295

tagcgtgcaa gctggtaaag gggaatgcct gtggcaagcc tcagccctgg gaatctgcac 1215

cgaggaagct ggtgcccagg gaggagcaga ggccgcgcat catggccagg tcagctgtga 1275

ggtctgagtg atctgcatgg tactggccag cctactcaag gtatcctaaa gtgcaagcag 1335

gcagagccac cctggggatg gacactggcc ctcctgtccc tggggaggcc ctctggggac 1395

tccctgccct gcataaaaag agggtgattt tctacttgtt gttatgtgtt tgctttcaaa 1455

ttgcttagta gtacctccat tcaagttatt atgagccagc ctcaagttag agagctaggc 1515

tcttcttcag gtggactctg cccaaatcac atacaagtca ggtggccatc aggggttttt 1575

ccaggccagg cctgtgacag gagatatggg agggggggtcg ggttagagct gggtttgttt 1635

ggatttttttg cgtttttttc ttcctgtatt tctgcttgaa gtgagaaaac ttgtctcctg 1695

tccaaccttt tctccataat tactgctgca cggtcgcctg ctgaccagtc acagtgacct 1755

cagacaccag aaggtgaggt ggcttattat gcccacactt tgtgttttgt tgtgagaata 1815

aacctttcca gactcccaaa aaaaaaaaaa aaaaaaaaaa aaaaaa                1861
```

<210> 3
<211> 2134
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (407)..(1303)

<400> 3
aattcggggt caaggcgaag ctcgcggggg gcgacagcga cggcggggag ctcctcgggg 60

agtaccccgg gatcccagag ctcagcgcgc tggaggacgt cgcgctcctg caggccccgc 120

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agccgcccgc ctgcaacgtg cacttcctgt cctcgctgct acccgcgcac cgcagcccgc 180

gggtgttttg cccctggggc gcctgggtcc tgcgaaggag cctcccaccc gggcgtccgc 240

atgatcccag ttgaaatcaa ggtaagcagg tggtactact acaagtaata atccggaaga 300

agcaactttg cagaatcttc ttgctcagga atcctgttgc aagttcccat ggtcccagga 360

actagaggat gcctcctgct gttctcttaa gaaagattcc aaccca atg gtg ata    415
                                                  Met Val Ile
                                                    1

tgc caa ttg aaa ggg ggc aca caa atg cta tgt ata gac aat tct aga   463
Cys Gln Leu Lys Gly Gly Thr Gln Met Leu Cys Ile Asp Asn Ser Arg
      5                  10                  15

aca aga gaa cta aaa gca ctc cat ttg gtt cct cag tat caa gat caa   511
Thr Arg Glu Leu Lys Ala Leu His Leu Val Pro Gln Tyr Gln Asp Gln
 20                  25                  30                  35

aat aat tat cta cag tca gat gtc cct aaa cca atg act gct tta gta   559
Asn Asn Tyr Leu Gln Ser Asp Val Pro Lys Pro Met Thr Ala Leu Val
                 40                  45                  50

ggg aga ttt ttg cca gca tca aca aaa tta aat ctc att aca caa caa   607
Gly Arg Phe Leu Pro Ala Ser Thr Lys Leu Asn Leu Ile Thr Gln Gln
             55                  60                  65

ctt gag gga gcc tta cca tcg gta gtc aac ggg tct gct ttc ccc tcg   655
Leu Glu Gly Ala Leu Pro Ser Val Val Asn Gly Ser Ala Phe Pro Ser
         70                  75                  80

gga tca act ctt cca gga cca cca aaa ata act ttg gct ggg tac tgt   703
Gly Ser Thr Leu Pro Gly Pro Pro Lys Ile Thr Leu Ala Gly Tyr Cys
     85                  90                  95

gac tgc ttt gcc agt ggg gac ttt tgc aac aac tgc aat tgt aat aat   751
Asp Cys Phe Ala Ser Gly Asp Phe Cys Asn Asn Cys Asn Cys Asn Asn
100                 105                 110                 115

tgt tgc aac aac ttg cat cat gat att gaa cgg ttt aaa gcc att aag   799
Cys Cys Asn Asn Leu His His Asp Ile Glu Arg Phe Lys Ala Ile Lys
                120                 125                 130

gca tgt ctt ggt aga aat cca gaa gct ttc cag cca aaa att ggg aag   847
Ala Cys Leu Gly Arg Asn Pro Glu Ala Phe Gln Pro Lys Ile Gly Lys
            135                 140                 145

ggc caa ttg ggc aat gtc aag ccc cag cac aac aaa ggg tgc aac tgc   895
Gly Gln Leu Gly Asn Val Lys Pro Gln His Asn Lys Gly Cys Asn Cys
        150                 155                 160

agg agg tca ggc tgc ctg aag aat tac tgc gag tgc tat gag gcc caa   943
Arg Arg Ser Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr Glu Ala Gln
    165                 170                 175
```

```
att atg tgt tct tct att tgc aaa tgc att ggt tgc aaa aat tat gaa   991
Ile Met Cys Ser Ser Ile Cys Lys Cys Ile Gly Cys Lys Asn Tyr Glu
180                 185                 190                 195

gaa agc cca gaa cga aag aca cta atg agc atg cca aac tac atg cag   1039
Glu Ser Pro Glu Arg Lys Thr Leu Met Ser Met Pro Asn Tyr Met Gln
                200                 205                 210

act gga ggt ttg gaa ggc agc cat tac ctg cca cca acg aaa ttt tca   1087
Thr Gly Gly Leu Glu Gly Ser His Tyr Leu Pro Pro Thr Lys Phe Ser
            215                 220                 225

gga ctt cca aga ttc agt cac gat agg cgg cct tcc tca tgc atc tcc   1135
Gly Leu Pro Arg Phe Ser His Asp Arg Arg Pro Ser Ser Cys Ile Ser
        230                 235                 240

tgg gag gtg gtg gag gcc aca tgc gcc tgc ctg ctt gct cag gga gaa   1183
Trp Glu Val Val Glu Ala Thr Cys Ala Cys Leu Leu Ala Gln Gly Glu
    245                 250                 255

gag gcc gag aaa gaa cac tgc tcc aag tgc ctg gca gag cag atg atc   1231
Glu Ala Glu Lys Glu His Cys Ser Lys Cys Leu Ala Glu Gln Met Ile
260                 265                 270                 275

ctg gag gaa ttt gga agg tgc tta tca cag att ctc cac act gag ttt   1279
Leu Glu Glu Phe Gly Arg Cys Leu Ser Gln Ile Leu His Thr Glu Phe
                280                 285                 290

aaa tct aag gga ttg aaa atg gag tagagtataa agtgtgaatg catgttgatt  1333
Lys Ser Lys Gly Leu Lys Met Glu
            295

ttgtcttagt ctagaaatct ctagtttaga aaggatgttt aggggaacat gaggctggct 1393

ctgcagcaac aaccaggctc ccctgcatcc ctgggcccag ggagtttact cagagctctc 1453

tgaagatgtg gcaacccatg ccccctttc tgaggaggtg catggcctga gcattgtttg 1513

tctggcccag aggagagagc ttgggttccc atagtcctgg gagagtgtct gcagggcggc 1573

ggagggcaga gcaggccctg cggagagctc actctggtcg actcttcctc tcagagaatg 1633

ttgctctgga ggctgctctg catgaaaacc ctaatggttt cttgtttgtt tttcaaatta 1693

tttagaaata agttctccgg atgggctgtt gtgataccac ttaaaatctc tagagaacta 1753

ctgaacacct aaagattttc tgtagcgtag atatttcccc agagacacgc gaactgtcag 1813

tctttcctaa ggcccccggg agacgcaggc aatggggcct cgcaggccag gcttgcacca 1873

gcatgtcttg agttagagga cttaaaatta tccagtttct tctgtgtttc tacttgaatt 1933

gtggaaaagc tctattatcc aattaacttc tccataatta ttgttgtaat attattattg 1993

tttgtaaaac atggttcaca taactagctt gtggaaacca gcaggtaaaa tgaattctta 2053
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agttgacgct tttggttctg ttgtaaagca aagatgaata aaaatttcca atgtcgaaaa 2113

aaaaaaaaaa aaaaaaaaaa a 2134

<210> 4
<211> 295
<212> PRT
<213> Mus musculus

<400> 4
Met Val Ile Cys Gln Leu Lys Gly Gly Ala Gln Met Leu Cys Ile Asp
1 5 10 15

Asn Cys Gly Ala Arg Glu Leu Lys Ala Leu His Leu Leu Pro Gln Tyr
20 25 30

Asp Asp Gln Ser Ser Phe Pro Gln Ser Glu Leu Pro Lys Pro Met Thr
35 40 45

Thr Leu Val Gly Arg Leu Leu Pro Val Pro Ala Lys Leu Asn Leu Ile
50 55 60

Thr Gln Val Asp Asn Gly Ala Leu Pro Ser Ala Val Asn Gly Ala Ala
65 70 75 80

Phe Pro Ser Gly Pro Ala Leu Gln Gly Pro Pro Lys Ile Thr Leu Ser
85 90 95

Gly Tyr Cys Asp Cys Phe Ser Ser Gly Asp Phe Cys Asn Ser Cys Ser
100 105 110

Cys Asn Asn Leu Arg His Glu Leu Glu Arg Phe Lys Ala Ile Lys Ala
115 120 125

Cys Leu Asp Arg Asn Pro Glu Ala Phe Gln Pro Lys Met Gly Lys Gly
130 135 140

Arg Leu Gly Ala Ala Lys Leu Arg His Ser Lys Gly Cys Asn Cys Lys
145 150 155 160

Arg Ser Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr Glu Ala Lys Ile
165 170 175

Met Cys Ser Ser Ile Cys Lys Cys Ile Ala Cys Lys Asn Tyr Glu Glu
180 185 190

Ser Pro Glu Arg Lys Met Leu Met Ser Thr Pro His Tyr Met Glu Pro
195 200 205

Gly Asp Phe Glu Ser Ser His Tyr Leu Ser Pro Ala Lys Phe Ser Gly
210 215 220

Pro Pro Lys Leu Arg Lys Asn Arg Gln Ala Phe Ser Cys Ile Ser Trp
225 230 235 240

Glu Val Val Glu Ala Thr Cys Ala Cys Leu Leu Ala Gln Gly Glu Glu
245 250 255

```
Ala Glu Gln Glu His Cys Ser Pro Ser Leu Ala Glu Gln Met Ile Leu
            260                 265                 270

Glu Glu Phe Gly Arg Cys Leu Ser Gln Ile Leu His Ile Glu Phe Lys
        275                 280                 285

Ser Lys Gly Leu Lys Ile Glu
    290                 295
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<210> 5
<211> 299
<212> PRT
<213> Homo sapiens

<400> 5

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Met Val Ile Cys Gln Leu Lys Gly Gly Thr Gln Met Leu Cys Ile Asp
  1               5                  10                  15

Asn Ser Arg Thr Arg Glu Leu Lys Ala Leu His Leu Val Pro Gln Tyr
            20                  25                  30

Gln Asp Gln Asn Asn Tyr Leu Gln Ser Asp Val Pro Lys Pro Met Thr
        35                  40                  45

Ala Leu Val Gly Arg Phe Leu Pro Ala Ser Thr Lys Leu Asn Leu Ile
     50                  55                  60

Thr Gln Gln Leu Glu Gly Ala Leu Pro Ser Val Val Asn Gly Ser Ala
 65                  70                  75                  80

Phe Pro Ser Gly Ser Thr Leu Pro Gly Pro Pro Lys Ile Thr Leu Ala
                85                  90                  95

Gly Tyr Cys Asp Cys Phe Ala Ser Gly Asp Phe Cys Asn Asn Cys Asn
            100                 105                 110

Cys Asn Asn Cys Cys Asn Asn Leu His His Asp Ile Glu Arg Phe Lys
        115                 120                 125

Ala Ile Lys Ala Cys Leu Gly Arg Asn Pro Glu Ala Phe Gln Pro Lys
    130                 135                 140

Ile Gly Lys Gly Gln Leu Gly Asn Val Lys Pro Gln His Asn Lys Gly
145                 150                 155                 160

Cys Asn Cys Arg Arg Ser Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr
                165                 170                 175

Glu Ala Gln Ile Met Cys Ser Ser Ile Cys Lys Cys Ile Gly Cys Lys
            180                 185                 190

Asn Tyr Glu Glu Ser Pro Glu Arg Lys Thr Leu Met Ser Met Pro Asn
        195                 200                 205

Tyr Met Gln Thr Gly Gly Leu Glu Gly Ser His Tyr Leu Pro Pro Thr
    210                 215                 220
```

Lys Phe Ser Gly Leu Pro Arg Phe Ser His Asp Arg Arg Pro Ser Ser
225 230 235 240

Cys Ile Ser Trp Glu Val Val Glu Ala Thr Cys Ala Cys Leu Leu Ala
245 250 255

Gln Gly Glu Glu Ala Glu Lys Glu His Cys Ser Lys Cys Leu Ala Glu
260 265 270

Gln Met Ile Leu Glu Glu Phe Gly Arg Cys Leu Ser Gln Ile Leu His
275 280 285

Thr Glu Phe Lys Ser Lys Gly Leu Lys Met Glu
290 295

<210> 6
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 6
ggagaatctg cgacaggcac 20

<210> 7
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 7
tccccagcca agttctcsgg 20

<210> 8
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 8
ttcattgacc tcaactacat g 21

<210> 9
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 9
gtggcagtga tggcatggac 20

<210> 10
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 10
tatgggcgcc tcctttcagc tgacaaat 28

<210> 11
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 11
actgaggaag cagatggagc gctttgag 28

<210> 12
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 12
tgactgaggg aaactgctct ggtcat 26

<210> 13
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 13
aacctgatgg ctggcttgat 20

<210> 14
<211> 20
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 14
tttttcttta ctttccttgg 20

<210> 15
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 15
ccgaagaagg gctccaagaa 20

<210> 16
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 16
tctccactca agacaagcct 20

<210> 17
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 17
tgggcccagg gagtttactc a 21

<210> 18
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 18
tctcccagga ctatgggaac ccaa 24

<210> 19
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 19
tcgaattcta tggtgatttg tcagctgaaa gga 33

<210> 20
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 20
gaattcgaat tcgcattccc ctttaccagc tt 32

<210> 21
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 21
accgtcgact gcctaaggtc ctgagaactt ggctgggga 39

<210> 22
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 22
Cys Leu Ser Gln Ile Leu His Ile Glu Phe Lys Ser Lys Gly Leu Lys
1 5 10 15

Ile Glu

<210> 23
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<220>
<221> MOD_RES
<222> (2)
<223> Arbitrary amino acid

<220>
<221> MOD_RES
<222> (4)..(12)
<223> Arbitrary amino acid

<220>
<221> MOD_RES
<222> (14)
<223> Arbitrary amino acid

<400> 23
Cys Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys
1 5 10 15

<210> 24
<211> 295
<212> PRT
<213> Mus sp.

<400> 24
Met Val Ile Cys Gln Leu Lys Gly Gly Ala Gln Met Leu Cys Ile Asp
1 5 10 15

Asn Cys Gly Ala Arg Glu Leu Lys Ala Leu His Leu Leu Pro Gln Tyr
20 25 30

Asp Asp Gln Ser Ser Phe Pro Gln Ser Glu Leu Pro Lys Pro Met Thr
35 40 45

Thr Leu Val Gly Arg Leu Leu Pro Val Pro Ala Lys Leu Asn Leu Ile
50 55 60

Thr Gln Val Asp Asn Gly Ala Leu Pro Ser Ala Val Asn Gly Ala Ala
65 70 75 80

Phe Pro Ser Gly Pro Ala Leu Gln Gly Pro Pro Lys Ile Thr Leu Ser
85 90 95

Gly Tyr Cys Asp Cys Phe Ser Ser Gly Asp Phe Cys Asn Ser Cys Ser
100 105 110

Cys Asn Asn Leu Arg His Glu Leu Glu Arg Phe Lys Ala Ile Lys Ala
115 120 125

Cys Leu Asp Arg Asn Pro Glu Ala Phe Gln Pro Lys Met Gly Lys Gly
130 135 140

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Arg Leu Gly Ala Ala Lys Leu Arg His Ser Lys Gly Cys Asn Cys Lys
145                 150                 155                 160

Arg Ser Gly Cys Leu Lys Asn Tyr Cys Glu Cys Tyr Glu Ala Lys Ile
                165                 170                 175

Met Cys Ser Ser Ile Cys Lys Cys Ile Ala Cys Lys Asn Tyr Glu Glu
            180                 185                 190

Ser Pro Glu Arg Lys Met Leu Met Ser Thr Pro His Tyr Met Glu Pro
        195                 200                 205

Gly Asp Phe Glu Ser Ser His Tyr Leu Ser Pro Ala Lys Phe Ser Gly
    210                 215                 220

Pro Pro Lys Leu Arg Lys Asn Arg Gln Ala Phe Ser Cys Ile Ser Trp
225                 230                 235                 240

Glu Val Val Glu Ala Thr Cys Ala Cys Leu Leu Ala Gln Gly Glu Glu
                245                 250                 255

Ala Glu Gln Glu His Cys Ser Pro Ser Leu Ala Glu Gln Met Ile Leu
            260                 265                 270

Glu Glu Phe Gly Arg Cys Leu Ser Gln Ile Leu His Ile Glu Phe Lys
        275                 280                 285

Ser Lys Gly Leu Lys Ile Glu
    290                 295
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